Supporting Information

Transformation of Pro-Leu-Gly-NH_2 Peptidomimetic Positive Allosteric Modulators of the Dopamine D_2 Receptor into Negative Modulators

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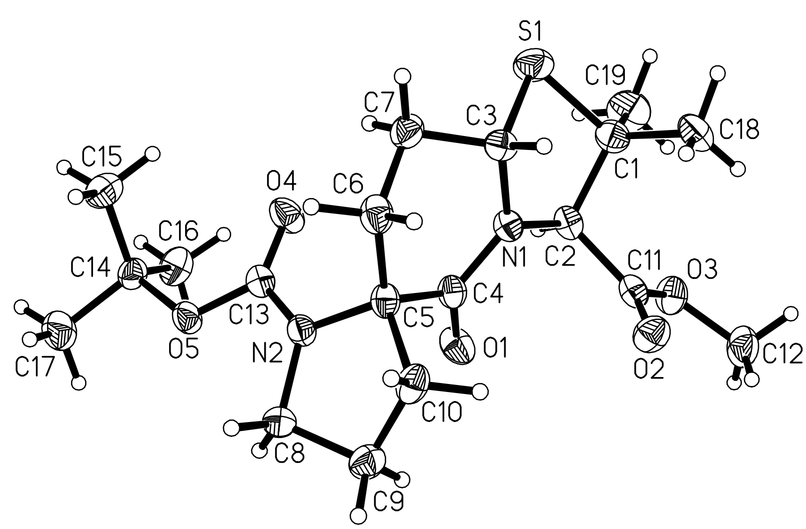


Figure 1. ORTEP drawing of the X-ray structure of **7b**.

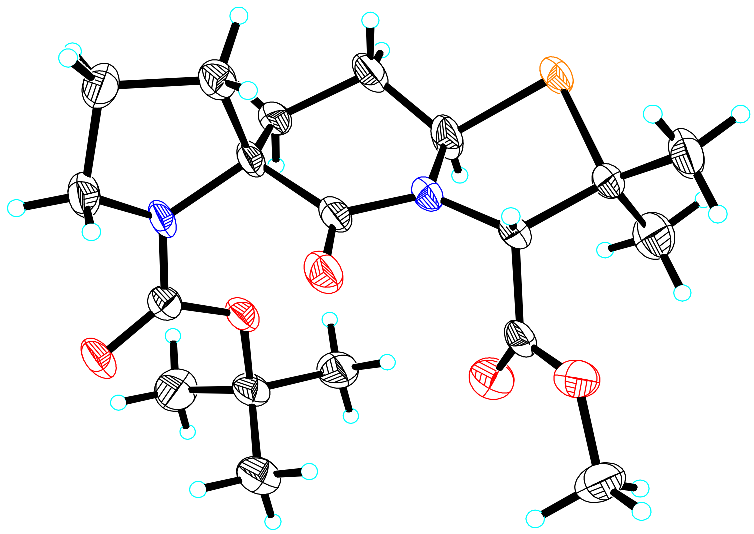


Figure 2. ORTEP drawing of the X-ray structure of **8a**.

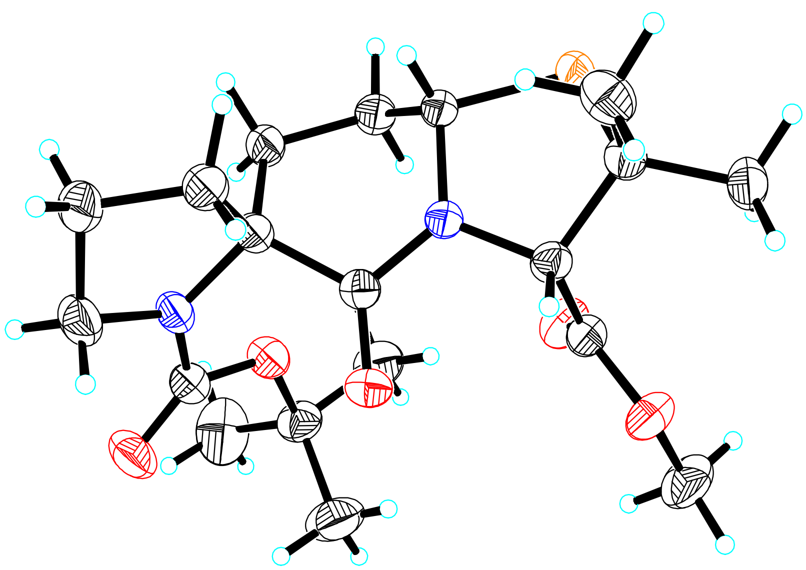


Figure 3. ORTEP drawing of the X-ray structure of **8b**.

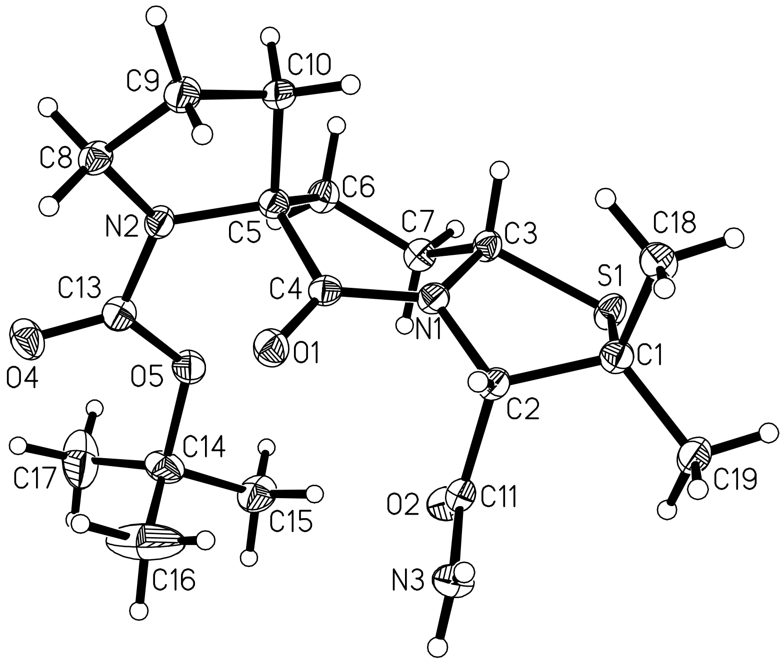


Figure 4. ORTEP drawing of the X-ray structure of **15b**.

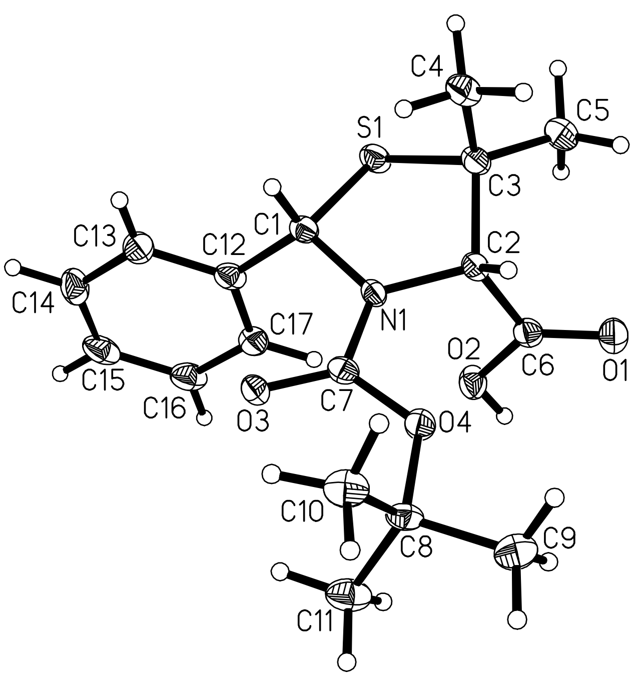
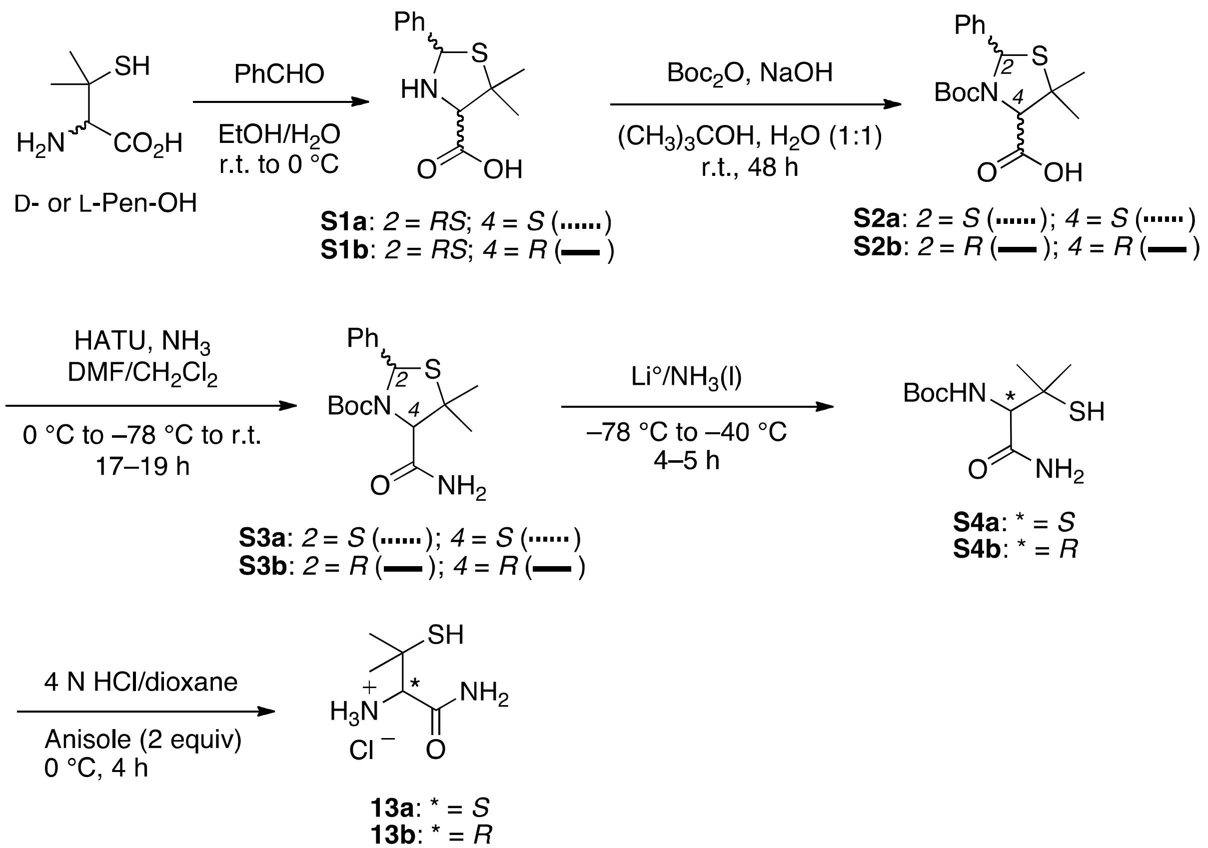


Figure 5. ORTEP drawing of the X-ray structure of **S2a**.

Synthesis of D- and L-Penicillamine Carboxamides 13a and 13b. As shown in Scheme S1, D- and L-penicillamine were condensed with benzaldehyde to give a diastereoisomeric mixture of the C-2-phenyl thiazolidines **S1a** and **S1b**, respectively. The diastereoisomers in each case appeared to be constantly inter-converting as observed by muted ^{13}C NMR intensities under ambient conditions. *N*-Boc protection of each diastereoisomeric mixture under prolonged reaction times led to the synthesis of the *N*-protected thiazolidine **S2a** or **S2b** as a single diastereoisomer; the *cis*-2,4-substituted thiazolidine. This was confirmed by X-ray structure determination of **S2a**[1] and there exists literature precedent for this observation with respect to thiazolidines derived from penicillamines.[2,3] It can be argued that although an equilibrium exists in the inter-conversion of the two diastereoisomeric forms in each case, the sterically less encumbered *cis*-form perhaps, reacts faster with Boc_2O to form the *N*-Boc derivative. This constantly pushes the equilibrium toward the *cis*-form and eventually leads to almost complete diastereospecific formation of **S2a** and **S2b**, respectively. Each acid was converted to the corresponding primary carboxamide, **S3a** or **S3b**, in the presence of ammonia and the coupling agent HATU. Dissolving metal reduction with lithium metal (Li°) in ammonia[4,5] led to the formation of the *N*-Boc penicillamine carboxamides **S4a** and **S4b**, respectively. *N*-Deprotection with 4 N HCl in dioxane in the presence of anisole as a cation scavenger gave the desired penicillamides **13a** and **13b**, respectively.[6]



Scheme S1. Synthesis of penicillamine carboxamides **13a** and **13b**.

(2*RS*, 4*S*)-5, 5-Dimethyl-2-phenylthiazolidine-4-carboxylic Acid (S1a). D-Penicillamine (746 mg, 5 mmol) was dissolved in 10 mL water with vigorous stirring. A solution of benzaldehyde (0.5 mL, 5 mmol) in 3 mL EtOH was added drop-wise to the aqueous solution. A thick-white precipitate formed almost immediately. Once addition was complete, the mixture was further diluted with EtOH-H_2O (4:1). This reaction was stirred vigorously for 1 h at rt and then for 4 h at 0 °C. The white colored precipitate was filtered and washed with water. The precipitate was then suspended in CH_2Cl_2 first and then xylenes and subjected to rotary evaporation in order to remove most of the water azeotropically. The slightly wet precipitate was dried under high vacuum to give the product **S1a** as a free-flowing white powder weighing 940 mg (79%). mp 165 °C (dec); TLC *Rf* 0.64 (iPrOH/NH_4OH, 4:1); ^1H NMR (400 MHz, DMSO-d_6, diastereoisomers present in a 1.5:1 ratio, major diastereomer indicated by *) δ 7.60–7.50 (m, 2H, aromatic CH), 7.45–7.20 (m, 6.3H, aromatic CH), 5.91 (s, 0.7H, 2-CH) , 5.69* (s,1H, 2-CH), 3.73* (s, 1H, 4-CH), 3.65 (s, 0.7H, 4-CH), 1.72* (s, 3H, CH_3), 1.60 (s, 2.1H, CH_3), 1.42* (s, 3H, CH_3), 1.36 (s, 2H, CH_3); ^{13}C NMR (100 MHz, DMSO-d$_6$, major diastereomer indicated by *) 170.7, 170.4* (C=O), 144.1, 139.1* (aromatic Cq), 128.5*, 126.2 (aromatic CH), 128.3*, 126.9 (aromatic CH), 128.2, 127.4* (aromatic CH), 74.1*, 73.2 (4-C), 69.1*, 67.7 (2-C), 60.1, 59.5* (5-C), 29.1*, 27.7, 28.2 *, 26.8 (CH$_3$)$_2$; ESI HRMS m/z 260.0718 [M + Na]$^+$, ($C_{12}H_{15}NO_2S$ + Na$^+$) requires 260.0716.

(2*RS*, 4*R*)-5,5-Dimethyl-2-phenylthiazolidine-4-carboxylic Acid (S1b). L-Penicillamine (460 mg, 3.08 mmol) was converted to 12b by the same procedure as describe above for **S1a**. The product was obtained as white flowing powder in a yield 533 mg (73%). mp 154-156 °C; TLC *Rf* 0.67 (iPrOH/NH_4OH, 4:1); ^{1}H NMR (400 MHz, DMSO-d$_6$, diastereoisomers present in a 1.4:1 ratio, major diastereomer indicated by *) δ 7.60–7.50 (m, 2H, aromatic CH), 7.45–7.20 (m, 6.3H, aromatic CH), 5.84 (s, 0.4H, 2-CH) , 5.62* (s,1H, 2-CH), 3.67* (s, 1H, 4-CH), 3.59 (s, 0.47H, 4-CH), 1.65* (s, 2.7H, CH3), 1.54 (s, 1.17, CH_3), 1.35* (s, 2.8H, CH_3), 1.30 (s, 1.3H,CH_3); ^{13}C NMR (100 MHz, DMSO-d$_6$, major diastereomer indicated by *) δ 170.6, 170.3* (C=O), 144.0, 139.0* (aromatic Cq), 128.4*, 128.2 (aromatic CH), 128.1*, 127.6 (aromatic CH), 127.3, 126.1* (aromatic CH), 74.0*, 73.1 (4-C), 69.0*, 67.6 (2-C), 60.0, 59.4* (5-C), 29.0*, 27.6, 28.1 *, 26.7 ((CH$_3$)$_2$); ESI HRMS m/z 260.0800 [M + Na]$^+$, ($C_{12}H_{15}NO_2S$ + Na$^+$) requires 260.0716.

(2*S*, 4*S*)-*N*-*tert*-Butyloxycarbonyl-5,5-dimethyl-2-phenylthiazolidine-4-carboxylic Acid (S2a). Sodium hydroxide (80 mg, 2 mmol) was added as a powder to a suspension of the amino acid **S1a** (475 mg, 2 mmol) in water (3 mL). Within 10 minutes, a clear solution was obtained. A solution of Boc$_2$O (445 mg, 2.04 mmol) in *tert*-butanol (3 mL) was added to the aqueous solution. The cloudy solution was stirred under Ar for 48 h during which time a white-colored suspension formed. *tert*-Butanol was removed by rotary evaporation and the residue diluted with H_2O. The pH of this solution was adjusted to 11–12 and it was washed with Et_2O. The pH of the basic solution was adjusted to 2–3 using HCl to give rise to a white precipitate, which was extracted into EtOAc (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give rise to the pure product as a white solid weighing 551 mg (82% yield) which was further crystallized using hexanes/EtOAc/2–3 drops of MeOH to give needle-shaped crystals. The product **S2a** was entirely a single diastereoisomer. mp 215–217 °C (dec); TLC *Rf* 0.60 (iPrOH/NH_4OH, 4:1); $[\alpha]_D$ –82.8 (*c* 1.0 $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (app d, 2H, Ar-C*H*), 7.35–7.24 (m, 3H, Ar-C*H*), 5.98 (br. s, 1H, 2-C*H*), 4.54 (s, 1H, 4-C*H*), 1.68 (s, 3H, 5-C(CH_3)$_2$), 1.47–1.55 (m, 12H, C(CH_3)$_3$, 5-C(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0 (acid *C*=O), 152.7 (carbamate *C*=O), 141.2 (Ar-*C*q), 127.7, 127.2, 126.6 (Ar-*C*H), 79.9 (*C*(CH_3)$_3$), 72.8 (4-*C*), 64.9 (2-*C*), 51.2 (5-*C*), 30.9 (5-C(CH_3)$_2$), 27.5 (C(CH_3)$_3$), 24.4 (5-C(CH_3)$_2$); ESI HRMS *m/z* 360.1243 $[M + Na]^+$, ($C_{17}H_{23}NO_4S + Na^+$) requires 360.1240.

(2*R*, 4R)-*N-tert*-Butyloxycarbonyl-5,5-dimethyl-2-phenylthiazolidine-4-carboxylic Acid (S2b). Compound **S1b** (510 mg, 2 mmol) was converted to **S2b** by the same procedure as describe above for **S2a**. The product was obtained as white powder in a yield 507 mg (72%) as a single diastereoisomer. mp 213–214 °C; TLC *Rf* 0.69 (iPrOH/NH_4OH, 4:1); $[\alpha]_D$ + 85.6 (*c* 1.0 $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6,) δ 12.89 (s, 1H, COOH), 7.69 (s, br, 2H, Ar-C*H*), 7.31–7.23 (m, 3H, Ar-C*H*), 6.02(d, 1H, 2-C*H*), 4.31 (s, 1H, 4-C*H*), 1.57 (s, 3H, 5-C(CH_3)$_2$), 1.31–1.03 (m, 12H, C(CH_3)$_3$, 5-C(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9 (acid *C*=O), 152.6 (carbamate *C*=O), 141.7, 141.1(Ar-*C*q), 127.7, 127.1, 126.5 (Ar-*C*H), 80.3, 79.9 (*C*(CH_3)$_3$), 72.7 (4-*C*), 64.8 (2-*C*), 51.4, 51.2 (5-*C*), 30.9, 29.7 (5-C(CH_3)$_2$), 27.5 (C(CH_3)$_3$), 25.9, 24.4 (5-C(CH_3)$_2$); ESI HRMS *m/z* 360.1240 $[M + Na]^+$, ($C_{17}H_{23}NO_4S + Na^+$) requires 360.1240.

(2*S*, 4S)-*N*-*tert*-Butyloxycarbonyl-5,5-dimethyl-2-phenylthiazolidine-4-carboxamide (S3a). A solution of the carboxylic acid **S2a** (337 mg, 1 mmol) in 2 mL anhydrous DMF was cooled to 0 °C. This was followed by the sequential addition of DIPEA (0.3 mL, 2 mmol) and HATU (456 mg, 1.2 mmol). The pale yellow-colored solution was stirred at this temperature for 20 min following which it was cooled to –78 °C. In a cooled measuring cylinder, 2 mL anhydrous ammonia was condensed into 5 mL anhydrous CH_2Cl_2. This solution was added to the former active ester resulting in a canary yellow precipitate formation almost immediately. An adapter fitted with a balloon was attached to the reaction flask. The reaction mixture was stirred at –78 °C for 7 h and then allowed to warm to room temperature over a 12 h period. The next morning, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and filtered to give a nearly colorless filtrate. The volatiles were removed by rotary evaporation, including the majority of DMF present and the residue was dissolved in EtOAc (60 mL) and washed sequentially with 10% citric acid, sat. $NaHCO_3$, and brine solutions. The organic layer, which was opalescent at this time, was dried over $MgSO_4$, filtered and concentrated to give colorless oil with some solid present. (approximately 80% pure by NMR). This material was further purified by flash chromatography with a gradient elution of 0–5% MeOH/CH_2Cl_2 to give the product **S3a** as a colorless oil (300 mg, 89% yield), which solidified under vacuum to give. mp 103–105 °C; TLC *Rf* 0.6 (CH_2Cl_2/MeOH, 20:1); $[\alpha]_D$ –75.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.6 Hz, 2H, ortho aromatic CH), 7.29–7.18 (m, 3H, meta and para aromatic CH), 6.32 (br. s, 1H, N*H*), 6.00 (s, 1H, 2-C*H*), 5.89 (br. s, 1H, N*H*), 4.3 (s, 1H, 4-C*H*), 1.56 (s, 3H, C(CH_3)$_2$), 1.37 (3H, C(CH_3)$_2$), 1.2 (s, 9H, Boc C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (amide *C*=O), 153.0 (carbamate *C*=O), 139.1 (aromatic *C*q), 127.8, 127.2, 125.7 (aromatic *C*H), 81.9 (*C*(CH_3)$_3$), 74.9 (4-*C*), 66.1 (2-*C*), 52.6 (5-*C*), 32.0 (5-C(*C*H_3)$_2$, 28.9 (C(*C*H_3)$_3$), 25.0 (5-C(*C*H_3)$_2$); ESI HRMS *m/z* 359.1405 $[M + Na]^+$ $C_{17}H_{24}N_2O_3S$+Na^+ requires 359.1400.

(2*R*, 4*R*)-*N*-*tert*-Butyloxycarbonyl-5,5-dimethyl-2-phenylthiazolidine-4-carboxamide (S3b). Compound **S2b** (505 mg, 1.5 mmol) was converted to **S3b** by the same method as describe above for **S3a**. The product was obtained as white foam in a yield 353 mg (70%). mp 117–119 °C; TLC *Rf* 0.86 (CH_2Cl_2/MeOH, 10:1); $[\alpha]_D$ 83.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.6 Hz, 2H, ortho aromatic CH), 7.33–7.24 (m, 3H, meta and para aromatic CH), 6.62(br s, 1H, N*H*), 6.48(s, 1H, 2-C*H*), 6.04 (br s, 1H, N*H*), 4.41 (s, 1H, 4-C*H*), 2.46 (s, 1H, SH), 1.61 (s, 3H, $C(CH_3)_2$), 1.25 (3H, $C(CH_3)_2$), 1.22 (s, 9H, Boc $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9 (amide *C*=O), 154.1 (carbamate *C*=O), 140.0 (aromatic *C*q), 128.4, 127.8, 126.5 (aromatic *C*H), 81.7 ($C(CH_3)_3$), 74.4 (4-*C*), 65.8 (2-*C*), 51.9 (5-*C*), 31.1 (5-$C(CH_3)_2$), 27.9 ($C(CH_3)3$), 24.0 (5-$C(CH_3)_2$); ESI HRMS *m/z* 359.1485 $[M + Na]^+$, ($C_{17}H_{24}N_2O_3S + Na^+$) requires 359.1400.

(*S*)-N-*tert*-Butyloxycarbonyl Penicillaminecarboxamide (S4a). The diastereomerically pure thiazolidine **S3a** (267 mg, 0.8 mmol) was placed in a 100 mL RBF that was then purged with Ar. The flask was cooled to –78 °C and equipped with an efficient teflon-coated stir bar. Approximately 25 mL anhydrous ammonia was directly condensed into the flask. The substrate dissolved in liquid ammonia even at this temperature. An adapter fitted with a balloon was then attached to the flask. Lithium metal was cut into 2 mm cubes and this was added to the reaction flask. After 2 min., the entire reaction mixture took on an intense royal blue color. The reaction was stirred vigorously at –78 °C for 2 h and then warmed to –40 °C for approximately 15 min. before being cooled back to –78 °C. The reaction was stirred for a further 2–3 h during which time Li metal was added to maintain the blue color. At the end of a total 5 h, the reaction was quenched by addition of solid NH_4Cl until the blue color disappeared and a white suspension was obtained. Ammonia was removed under a gentle stream of Ar over 20 min to leave behind a free-flowing chalky white solid. The solid was partitioned between water (pH adjusted to 7) and

CH_2Cl_2 (60 mL each). The aqueous layer was washed 2 more times with 70 mL portions of CH_2Cl_2. The organic layers were pooled together, dried over $MgSO_4$, filtered and concentrated to give a colorless oil/foam with was 90% pure by NMR analysis. It was further purified by flash chromatography with a gradient elution consisting of 0–10% MeOH/CH_2Cl_2 to give the product **S4a** as a white foam/solid weighing 145 mg (73%). mp 55–57 °C; TLC *Rf* 0.58 (CH_2Cl_2/MeOH, 10:1); $[\alpha]_D$ –10.4 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (br s, 1H, NH_2), 5.74 (br s, 1H, NH_2), 5.62 (d, *J* = 8 Hz, 1H, Boc N*H*), 4.13 (d, *J* = 9.2 Hz, 1H, α-C*H*), 1.54 (s, 3H, S-C(CH_3)$_2$), 1.44 (Boc C(CH_3)$_3$), 1.34 (s, 3H, S-C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9 (amide *C*=O), 154.8 (carbamate *C*=O), 80.5 (*C*(CH_3)$_3$), 62.3 (α-*C*), 46.4 (β-*C*), 31.9 (C(*C*H_3)$_2$), 29.3 (Boc C(*C*H_3)$_3$); ESI HRMS *m/z* 271.1091 $[M + Na]^+$, ($C_{10}H_{20}N_2O_3S + Na^+$) requires 271.1087.

(*R*)-N-*tert*-Butyloxycarbonyl Penicillaminecarboxamide (S4b). Compound **S3b** (672 mg, 2.0 mmol) was converted to **S4b** by the same procedure as describe above for **S4a**. The product was obtained as a white foam in a yield of 347 mg (70%). mp 59-62 °C; TLC *Rf* 0.56 (CH_2Cl_2/MeOH, 10:1); $[\alpha]_D$ 10.5 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (br.s, 1H, NH_2), 5.94 (br.s, 1H, NH_2), 5.59 (d, *J* = 9.24 Hz, 1H, Boc N*H*), 4.11 (d, *J* = 9.28 Hz, 1H, α-C*H*), 2.39 (s, 1H, SH), 1.46 (s, 3H, S-C(CH_3)$_2$), 1.37 (s, 9H, Boc C(CH_3)$_3$), 1.28 (s, 3H, S-C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (amide *C*=O), 155.8 (carbamate *C*=O), 80.3(*C*(CH_3)$_3$), 61.7 (α-*C*), 45.7 (δ*C*), 30.9 (C(*C*H_3)$_2$), 28.4 (C(*C*H_3)$_2$), 28.3 (Boc C(*C*H_3)$_3$); ESI HRMS *m/z* 271.1121 $[M + Na]^+$, ($C_{10}H_{20}N_2O_3S + Na^+$) requires 271.1087.

D-Penicillaminecarboxamide Hydrochloride (13a). The Boc-protected penicillamine-carboxamide **S4a** (145 mg, 0.6 mmol) was dissolved in 4 N HCl/dioxane (8 mL) and the solution cooled to 0 °C. While the solution was vigorously stirred, anisole (0.2 mL, 2.4 mmol) was added dropwise to the solution. The reaction mixture was stirred until the starting material had been completely consumed as determined by periodic analysis by TLC. At the end of 4 h, when all the starting material was consumed, the volatiles were removed by rotary evaporation. The white solid residue was triturated four times with hot Et_2O and the organic supernatant layer decanted each time to remove most of the anisole. The residue was dried under vacuum to give the pure product **13a** weighing 110 mg (quantitative yield) as a free-flowing powder. mp > 210 °C (dec); TLC *Rf* 0.73 (iPrOH/NH_4OH, 4:1); $[\alpha]_D$ –30.5 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) 3.84 (s, 1H, α-C*H*), 1.48, 1.39 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (100 MHz, CD_3OD) 167.7 (*C*=O), 63.2 (α-*C*), 44.8 (β-*C*), 31.0, 28.64. (C(*C*H_3)$_2$), ESI HRMS *m/z* 149.1234 $[M + H]^{+,}$ ($C_5H_{13}N_2OS$ + H^+) requires 149.0743.

L-Penicillamine carboxamide Hydrochloride (13b). Compound **S4b** (672 mg, 2.0 mmol) was converted to **13b** by the same procedure as describe above for **13a**. The product was obtained as a free-flowing powder in a yield 430 mg (97%). Mp. 212 °C (dec); TLC *Rf* 0.69 (iPrOH/NH4OH, 4:1); $[\alpha]_D$ 28.1 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 2H, NH_2), 8.11 (s, 1H, NH), 7.67 (s, 1H, NH) 3.86 (s, 1H, α-C*H*), 1.48, 1.40 (d, 6H, J = 2.0 Hz, C(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2 (*C*=O), 61.9 (α -*C*), 44.4 (β -*C*), 29.9, 29.33 (C(*C*H_3)$_2$); ESI HRMS *m/z* 149.0749 $[M + H]^+$, ($C_5H_{13}N_2OS$ + H^+) requires 149.0962.

References

1. CCDC 831952 contains the supplementary crystallographic data for compound **S2a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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